EXHIBIT A

Role of the enteric nervous system in the control of migrating spike complexes in the feline small intestine

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De Vos, Wayne C. Role of the enteric nervous system in the control of migrating spike complexes in the feline small intestine. *Am. J. Physiol.* 265 (*Gastrointest. Liver Physiol.* 28): G628–G637, 1993.—The effects of agonists and antagonists of nicotinic, muscarinic (M_1 and M_2), and adrenergic receptors on migrating spike complexes (MSC) in ileum of fasting cats are reported. Hexamethonium decreased MSC frequency and blocked propagation. Atropine at low concentrations increased MSC frequency and increased velocity of propagation; atropine at high concentration blocked propagation. Pirenzepine (Pz; M_1 antagonist) increased MSC frequency and propagation velocity. McNeil A-343 (M_1 agonist), by a Pz-sensitive phentolamine-insensitive mechanism, and 4-diethylamine-methylpiperidine (4-DAMP; M_2 antagonist) blocked propagation of an ongoing MSC but had no significant effect on frequency or velocity. Bethanechol (M_2-receptor agonist) increased phasic spiking by a 4-DAMP-sensitive mechanism and blocked MSC propagation by a Pz-sensitive mechanism. Phenylephrine (α_1-adrenoceptor agonist) or oxymetazoline (α_2-adrenoceptor agonist) blocked MSC propagation but had no effect on MSC frequency or velocity. Phentolamine (nonselective α_1-adrenoceptor antagonist), prazosin (α_1-adrenoceptor antagonist), or yohimbine (α_2-adrenoceptor antagonist) alone had no effect on MSC activity. The conclusion is that the enteric nervous system controls and regulates the MSC by the following proposed mechanisms. *1*) M_1-muscarinic receptors, located either on postganglionic inhibitory neurons or presynaptically at a nicotinic synapse and/or neuromuscular junction, are involved in the tonic inhibitory control of MSC initiation and propagation. *2*) Nicotinic and M_2 muscarinic receptors, located on excitatory postganglionic motoneurons and smooth muscle cells, respectively, are important in the initiation and/or propagation of MSC. *3*) α_1-Adrenoceptors on the smooth muscle cells and α_2-adrenoceptors located presynaptically at the nicotinic ganglionic synapses are not tonically active but inhibit MSC activity (4). Smooth muscle β-adrenoceptors do not play a significant role in neural control of MSC activity.

nicotinic receptor; muscarinic receptor; adrenergic receptor; fasting cats

THE MAMMALIAN SMALL INTESTINE during the fasting state is a versatile organ, expressing a variety of myoelectric activities. In the previous paper, a classification of propagated myoelectric phenomena was proposed that categorizes activities based on whether or not they are correlated with slow waves (9). Representative of the first category is the migrating myoelectric complex (MMC). The small intestine of the fasting cat lacks a slow wave-associated complex; rather, it has the migrating spike complex (MSC), an hourly propagated burst of spike potentials and associated contraction that migrates aborad at a rate of ~1–8 mm/s, accelerating caudally, reducing, and attenuating slow-wave frequency and shape as it passes (4, 9, 30).

The role of the enteric nervous system has been examined for the MMC and the giant migrating complex (GMC), but not for the MSC. This paper *1*) characterizes the role of nicotinic, muscarinic, and adrenergic receptors in the control and modulation of the MSC and *2*) suggests a neural circuit for origination and propagation of MSCs. Some of this work has previously been published in abstract form (10, 11).

MATERIALS AND METHODS

Ten healthy cats (3.5–5.5 kg) of either sex were implanted with a set of four bipolar electrodes. Surgical protocol has been previously described (9). Chronic indwelling intraperitoneal cannulas (for hexamethonium and atropine infusion) or intravenous femoral catheters (for all other pharmacological agents) were also implanted. Intraperitoneal cannulas consisted of Silastic tubing (Dow Corning, 0.030 × 0.065 in.); intravenous catheters consisted of Tygon tubing (Norton Plastics, 0.04 × 0.07 in.) with an attached 10-cm tip of PE-60 tubing (Intramedic, 0.03 × 0.048 in.) joined to the Tygon tubing. The distal tip of the intraperitoneal cannula was anchored to the intestinal serosa at the second electrode position using 2-0 nonabsorbable monofilament suture. The proximal end of the cannula/catheter was tunneled subcutaneously to the midscapular region, exteriorized through a stab incision, and attached to a three-way stopcock. The electrode miniconnector and three-way stopcock were both placed in pockets of a nylon mesh jacket (Alice King Medical Arts, Chatham, CA) worn at all times by the cat.

Each cat was allowed to recover for a minimum of 7 days before experiments began. All cats recovered uneventfully and remained healthy; catheters were periodically flushed with heparinized saline (500 U/ml). The cats were used for an average of 8 mo (range, 2–14 mo).

The experimental protocol for recording has been previously described (9). Infusion of drugs took place only after at least one MSC had been recorded. When studying the effect of a drug on MSC propagation, following the occurrence of at least one MSC, the infusion took place after the next MSC had passed the first electrode position (E_1) but before it had reached E_2. Recording was then continued for at least 4 h.

Pharmacological compounds used were the following: hexamethonium, carbamyl-β-methylcholine chloride (bethanechol), phentolamine, phenylephrine, oxymetazoline, yohimbine, isoproterenol, and timolol (Sigma); atropine sulfate (Butler); pirenzepine (a gift from Boehringer-Ingelheim); 3-*M*-chlorophenyl-carbamoyloxy-2-butyryltrimethylammonium chloride (McNeil A-343; Research Biochemical), 4-diethylamine-methylpiperidine (4-DAMP; a gift from R. B. Barlow); and prazosin (a gift from Pfizer). Drugs were dissolved in 0.9% saline and stored at 4°C except pirenzipine, McNeil A-343, and 4-DAMP, which were suspended in saline and separated into vials in 2-ml aliquots, each containing 250 or 500 μg drug, lyophilized, and stored at −10°C. Prazosin and yohimbine were dissolved over heat in a 10% dimethyl sulfoxide saline solution and stored at 4°C.

Recorded electrical activity was visually tabulated and statistically analyzed as described previously (9).

RESULTS

Normal Fasting Activity

As described previously, ~20 h after a meal, irregular cycling of the MSC began. The majority of recorded MSCs involved all four electrode sites; MSCs appeared first at the most oral electrode position and rarely failed

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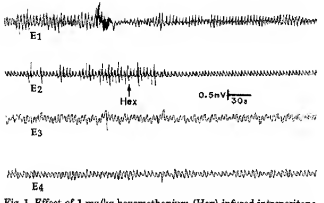

Fig. 1. Effect of 1 mg/kg hexamethonium (Hex) infused intraperitoneally near second electrode position (arrow) after migrating spike complex (MSC) appearance at E_1. Hex blocked MSC propagation and inhibited MSC activity for a significant length of time.

to migrate past the lower electrode positions. The average MSC period ($n = 10$ cats) was 51.2 ± 2.8 min, and propagation velocity increased aborally such that the MSC was traveling an average of three times faster between the distal two electrode positions relative to the proximal two electrode positions.

Effect of Nicotinic Receptor Blockade

Hexamethonium (1–2 mg/kg) infused intraperitonially after the appearance of an MSC at E_1 was followed by blockade of further propagation in eight of nine trials in five cats (Figs. 1 and 2*B*). In contrast, saline infusion never blocked an ongoing MSC ($n = 10$ in 5 cats; Fig. 2*A*). The first MSC following hexamethonium infusion occurred an average of 272.7 ± 23.9 min after the blocked MSC. This was significantly longer than after saline infusion ($P < 0.01$) (Table 1). When MSC activity returned after the period of inhibition, the average MSC period and propagation velocity were not significantly different from control values.

Effect of Muscarinic Block by Atropine

Atropine was infused both intraperitoneally and intravenously. Intraperitoneal infusion of atropine after the migration of an MSC past the first electrode position elicited a dose-dependent dual response. At all three doses (1, 10, and 50 μg/kg), atropine infusion was followed by a significant increase in MSC activity; the intercomplex period was shortened and velocity of propagation was increased (Fig. 2, *C–E*). After the low doses (1 and 10 μg/kg), MSC excitation persisted for at least 6 h. After the highest dose (50 μg/kg), a period of MSC inhibition followed the early excitation. The inhibition lasted an average of 167.7 ± 10.2 min (Table 1). When MSC activity returned, it occurred with a significantly increased frequency and propagation velocity, similar to that observed following the lower doses. Intravenous infusion of 50 μg/kg atropine ($n = 3$) was followed by an immediate inhibition of MSC propagation; this blockade was followed by a period of inhibition and postinhibitory excitation similar to that following the intraperitoneal infusion of 50 μg/kg atropine.

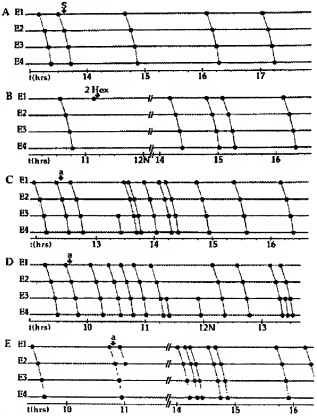

Fig. 2. Representative experiments showing effect of various treatments on propagation and frequency of MSC activity. MSC events are represented by connected circles. In all experiments, bolus intraperiteneal infusion occurred as MSC passed between electrode positions 1 (E_1) and 2 (E_2). *A*: saline infusion (*S*) had no significant effect on MSC propagation or frequency. *B*: 1–2 mg/kg Hex blocked MSC propagation and inhibited MSC activity for a significant period. MSC frequency and propagation velocity after inhibition were not significantly different than after saline. *C* and *D*: 1 μg/kg atropine (a) did not block MSC propagation and was followed by a significant increase in MSC frequency compared with saline. *E*: 50 μg/kg atropine did not block MSC propagation but was followed by a period of inhibition of MSC activity. MSC frequency after inhibition was significantly increased compared with saline.

Effect of Muscarinic Receptor Subtype-Selective Agents

M_1-receptor blockade. The M_1-selective antagonist pirenzepine (Pz) was infused intravenously at 1, 10, or 75 μg/kg; the intestinal response was dose dependent, showing a low-dose/excitatory, high-dose/inhibitory profile. Infusion of Pz at low doses of 1 and 10 μg/kg failed to block MSC propagation and was followed by an immediate increase in MSC activity (Fig. 3, *B* and *C*). The first MSC period after 1 or 10 μg/kg Pz infusion (4.4 ± 0.7 and 3.0 ± 0.6 min, respectively) was significantly less than the same period following saline ($P < 0.05$; Table 2). The average MSC period for the 6 h following infusion was also significantly decreased after both 1 and 10 μg/kg Pz compared with saline infusion (Table 2). Propagation velocity of the MSC was increased following both doses, although this increase reached significance only after the 10 μg/kg dose (Table 2). Figure 4 shows a typical example of increased MSC activity observed immediately after infusion of 1 μg/kg Pz. Effects of 1 and 10 μg/kg Pz are graphically shown in Fig. 3, *B* and *C*, respectively. Figure

Table 1. *Comparison of saline, hexamethonium, and atropine effect on MSC occurrence and propagation velocity after intraperitoneal infusion*

	n	Time to Next MSC After Infusion, min	Duration of MSC Inhibition, min	MSC Period After Infusion or Inhibition, min	Propagation Velocity, mm/s		
					E_1-E_2	E_2-E_3	E_3-E_4
Saline	10	46.4±10.7	46.4±10.7	56.4±8.3	1.4±0.2	2.1±0.4	5.6±1.4
Hexamethonium (1-2 mg/kg)	10		272.7±23.9*	47.3±5.5	1.4±0.2	2.0±0.3	5.1±1.1
Atropine							
1 μg/kg	10	29.3±8.0		29.1±3.7*	1.5±0.3	2.4±0.4	6.0±1.3
10 μg/kg	12	15.4±2.8*		24.0±2.7*	1.7±0.3	2.7±0.7	6.7±1.6
50 μg/kg	9	5.7±1.4†	169.7±10.2*	18.6±1.7†	1.6±0.3	2.5±0.5	6.4±1.3

Values are means ± SE; n, number of trials analyzed. Values for average time to next migrating spike complex (MSC) after infusion are for those drugs that did not inhibit propagation of an ongoing MSC. When inhibition of MSC propagation was observed after infusion, interval between blocked MSC and next MSC to occur was calculated as average duration of MSC inhibition. Saline values for first two columns are derived from same MSC interval, that is, first interval after saline infusion. If drug treatment was followed by inhibition of an ongoing MSC, average MSC period (3rd column) was determined beginning with first MSC to occur after inhibition. * $P < 0.05$ compared with saline control. † $P < 0.01$ compared with saline control.

34 is a saline control. A high dose of Pz (75 μg/kg) initially blocked ongoing propagation, and MSC activity was inhibited for a period significantly longer than the first MSC period following saline ($P < 0.05$; Table 2). MSCs following recovery from the inhibitory period occurred at a significantly increased frequency ($P < 0.05$; Table 2). MSC propagation velocity was also increased by Pz, although this increase reached significance only between E_2 and E_3 ($P < 0.05$) (Table 2). A typical intestinal response to 75 μg/kg Pz is shown graphically in Fig. 3*D*.

M_3-receptor blockade. The M_3-selective antagonist 4-DAMP, infused intravenously at 10 or 50 μg/kg during the passage of an MSC, blocked MSC propagation only at the higher dose (Fig. 3*E*). Neither the nonblocking dose of 10 μg/kg nor the blocking dose of 50 μg/kg was followed by a significant delay in occurrence of the next MSC compared with the same interval following saline (Table 2). The average MSC period and velocity of propagation following both 10 and 50 μg/kg 4-DAMP were not significantly altered (Table 2).

M_1-receptor stimulation. Intravenous infusion of the M_1-selective agonist McNeil A-343 at 100 μg/kg during the passage of an MSC immediately blocked further propagation. This blockade was never observed following a lower dose of 25 μg/kg. MSC inhibition by 100 μg/kg McNeil A-343 was not blocked by the presence of 0.5 mg/kg phentolamine (α dose found to block α-receptors) ($n = 5$). McNeil A-343 (100 μg/kg) infused during an MSC induced by 10 μg/kg Pz ($n = 5$) failed to block propagation. Neither 25 nor 100 μg/kg McNeil A-343 infusion was followed by a delay in occurrence of the next MSC following infusion compared with saline (Table 2); also, the average MSC period and propagation velocity following either dose of McNeil A-343 were not significantly altered relative to saline (Table 2).

M_2-receptor stimulation. The M_2-selective agonist bethanechol, infused intravenously during an MSC at 10–30 μg/kg, was followed by an immediate blockade of MSC propagation as well as an increase in phasic spiking ($n = 6$) (Fig. 5*A*). A prolonged decrease in slow-wave frequency

Table 2. *Comparison of pirenzepine, 4-DAMP, and McNeil A-343 effect on MSC occurrence and propagation velocity after intravenous infusion*

	Time to Next MSC After Infusion, min	Duration of MSC Inhibition, min	MSC Period After Infusion or Inhibition, min	Propagation Velocity, mm/s		
				E_1-E_2	E_2-E_3	E_3-E_4
Saline	51.7±16.1	51.7±16.1	54.7±13.2	1.6±0.1	2.4±0.1	4.8±0.2
McN A-343						
25 μg/kg	79.9±6.6		56.9±7.7	1.6±0.1	2.5±0.1	4.5±0.3
100 μg/kg		54.8±17.7	55.3±7.6	1.6±0.1	2.5±0.1	4.5±0.3
Pirenzepine						
1 μg/kg	4.4±0.7*		23.2±1.6*	1.8±0.1	2.8±0.2	4.6±0.3
10 μg/kg	3.0±0.6*		9.7±1.5†	2.3±0.3*	3.5±0.3	6.4±0.4
75 μg/kg		128.6±16.6*	18.8±2.4*	2.2±0.2	3.3±0.2	5.9±0.7
4-DAMP						
10 μg/kg	57.7±12.5		42.3±5.2	1.6±0.1	2.3±0.1	4.3±0.1
50 μg/kg		79.0±6.3	36.7±3.2	1.6±0.1	2.6±0.1	4.8±0.3
Bethanechol (10–30 μg/kg)			49.4±9.8	1.9±0.1	2.5±0.1	4.5±0.5

Values are means ± SE of 10 trials analyzed. 4-DAMP, 4-diethylamine-methylpiperidine. Values for average time to next MSC after infusion are for those drugs that did not inhibit propagation of an ongoing MSC. When inhibition of MSC propagation was observed after infusion, interval between blocked MSC and next MSC to occur was calculated as average duration of MSC inhibition. Saline values for first 2 columns are derived from same MSC interval, that is, first interval after saline infusion. If drug treatment was followed by inhibition of an ongoing MSC, average MSC period (3rd column) was determined beginning with the first MSC to occur after inhibition. * $P < 0.05$ compared with saline control. † $P < 0.01$ compared with saline control.

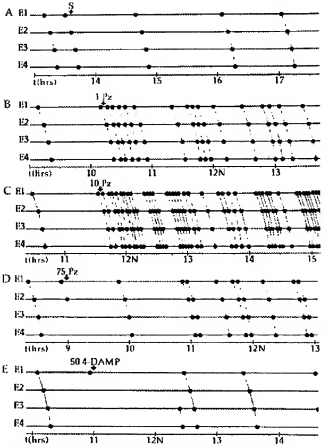

Fig. 3. Representative experiments showing effect of saline, M_1- or M_2-muscarinic receptor antagonist infusion on the propagation and frequency of MSC activity. MSC events are represented by connected circles. In all experiments, bolus intravenous infusion occurred as MSC passed between E_1 and E_2. *A*: saline infusion (S) had no significant effect on MSC propagation or frequency. *B* and *C*: 1 μg/kg pirenzepine (Pz) did not block MSC propagation but significantly increased MSC frequency. *D*: 75 μg/kg Pz consistently blocked MSC propagation and significantly increased MSC frequency after reappearance of MSC activity. *E*: 50 μg/kg 4-diethylamine-methylpiperidine (4-DAMP) consistently blocked MSC propagation but had no significant effect on MSC frequency or propagation velocity after reappearance of MSC activity.

was also observed (Fig. 6*A*). Lower doses of bethanechol (2–10 μg/kg, $n = 6$) did not block MSC propagation but were followed by an increase in phasic spiking. In seven experiments at 10–30 μg/kg, the spiking induced by bethanechol lasted an average of 33.2 ± 1.3 min.

To determine whether bethanechol was acting selectively at M_2 receptors to block MSC propagation and increase phasic spiking, 4-DAMP (10 μg/kg) or Pz (10 μg/kg) was administered before bethanechol infusion. When 4-DAMP was infused just before bethanechol during an MSC ($n = 6$), bethanechol blocked MSC propagation but induced little phasic spiking (Fig. 5*B*) and did not decrease slow-wave frequency (Fig. 6*B*). Bethanechol infused during a Pz-induced MSC ($n = 6$) failed to block propagation but was followed by an increase in spiking (Fig. 7).

To verify that MSC propagation blockade by bethanechol was not by way of a reflex increase in systemic sympathetic output secondary to a muscarinic receptor-mediated decrease in heart rate, 0.5 mg/kg phentolamine was infused before bethanechol in six experiments. In all cases, α-receptor blockade by phentolamine did not inhibit bethanechol-induced inhibition of MSC propagation or increase in phasic spiking.

The effect of M_1-receptor stimulation on bethanechol-induced phasic spiking was examined (Fig. 8). In six trials in four animals, following infusion of an MSC-blocking dose of bethanechol, 100 μg/kg McNeil A-343 was infused during the resulting increased phasic spiking between 5 and 15 min after bethanechol infusion. Immediately after the McNeil A-343 infusion, phasic spiking was reduced or completely abolished for an average of 27.3 ± 5.3 s (range, 15–50 s). This inhibition was not blocked by prior administration of 0.5 mg/kg phentolamine ($n = 3$) but could be blocked by prior administration of 10 μg/kg pirenzepine ($n = 3$).

Effect of Adrenoceptor Subtype-Selective Agonists and Antagonists

α-Adrenoceptor blockade. Phentolamine (0.5–1.0 mg/kg), a dose which increased heart rate and elicited relaxation of the nictitating membranes, had no significant effect on propagation velocity or occurrence of MSC activity (Table 3).

α-Adrenoceptor subtype stimulation. α_1-Adrenoceptor stimulation by 0.1 mg/kg phenylephrine consistently blocked MSC propagation but did not significantly delay the occurrence of the next MSC ($n = 6$; Table 3). Infusion of 0.03 mg/kg phenylephrine failed to block an ongoing MSC ($n = 3$). The inhibition of MSC propagation by phenylephrine was blocked by the α_1-adrenoceptor antagonist prazosin (1 μg/kg, $n = 4$) but not by α_2-adrenoceptor antagonist yohimbine (0.1–0.3 mg/kg, $n = 4$).

α_2-Adrenoceptor stimulation by 1–3 μg/kg oxymetazoline during the passage of an MSC blocked further propagation of the MSC but did not significantly delay the occurrence of the next MSC following infusion ($n = 4$; Table 3). Infusion of a lower dose (0.3 μg/kg) of oxymetazoline failed to block MSC propagation ($n = 3$). The inhibition of MSC propagation by oxymetazoline was blocked by yohimbine (0.1–0.3 mg/kg, $n = 4$) but not by prazosin (1 μg/kg, $n = 2$).

β-Receptor blockade. Timolol (1.0 μg/kg) infused intravenously during the passage of an MSC had no significant effect on MSC propagation, insignificantly decreased the first MSC period following infusion, and did not significantly change MSC frequency or propagation velocity (Table 3). This dose of timolol was found to suppress an increase in heart rate observed following infusion of isoproternol ($n = 4$).

β-Receptor stimulation. Intravenous infusion of 30 μg/kg isoproternol, a dose observed consistently to increase heart rate, failed to block propagation of the MSC ($n = 6$). Also, the occurrence of the next MSC was not significantly delayed, and the average MSC period following isoproternol was not significantly increased compared with saline (Table 3). Propagation velocity of the MSC activity following isoproternol was also not changed significantly (Table 3).

DISCUSSION

Characterization of two basic patterns of migrating intestinal phenomena (i.e., slow wave associated and slow

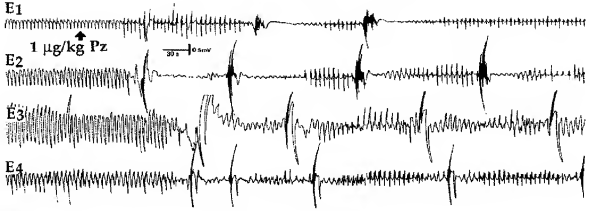

Fig. 4. Effect of low-dose Pz on occurrence of MSC activity. Pz (1 μg/kg) was infused intravenously at arrow and was followed by a sustained increase in MSC frequency.

wave independent) (17) during the last 20 yr has included investigations of the roles played by the intrinsic and extrinsic nerves in their control and modulation. The MMC, a slow wave-associated phenomenon observed in all mammals except the cat, requires nicotinic and muscarinic receptors for its expression (26, 27); also, M_1-muscarinic receptors are involved in the inhibitory control of the MMC (51). The GMC (also called prolonged propagated contractions or power contractions), a slow wave-independent activity observed in the rat, dog, and human small intestine (17, 18), requires nicotinic, opioid, and possibly muscarinic receptors for its expression (17). The migrating action potential complex (MAPC), a slow wave-independent activity observed in the rabbit ileum, requires nicotinic and muscarinic receptors (21).

In this paper, pharmacological evidence has been given for the neural circuitry involved in the control and modulation of the MSC. The roles of nicotinic, M_1- and M_2-muscarinic receptors, α_1- and α_2-adrenoceptors, and β-adrenoceptors were investigated. Based on the results, a

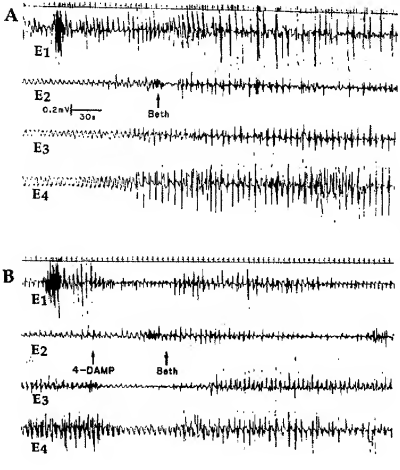

Fig. 5. *A*: effect of bethanechol (Beth) on electrical activity during fasting. Bethanechol (20 μg/kg) infused after appearance of the MSC at E_2 was followed by an immediate increase in spiking, decrease in slow-wave frequency, and blockade of MSC propagation. *B*: effect of 4-DAMP on activity induced by bethanechol. In presence of 10 μg/kg 4-DAMP (a dose which does not block MSC propagation), 10 μg/kg bethanechol infusion still blocked MSC propagation; however, less phasic spiking activity was observed.

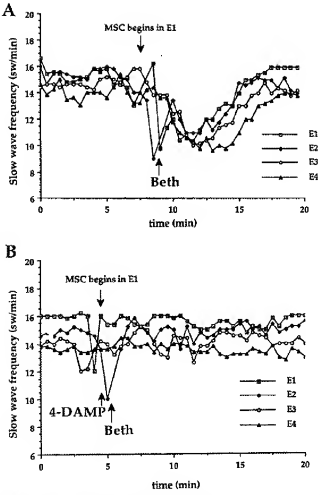

Fig. 6. Effect of bethanechol (Beth) on slow-wave frequency. *A*: 20 μg/kg bethanechol was infused after appearance of an MSC at E_1. MSC propagation was inhibited, and slow-wave frequency was transiently decreased at all 4 electrode sites. *B*: 10 μg/kg 4-DAMP, infused immediately before infusion of 20 μg/kg bethanechol during passage of an MSC, inhibited effect of bethanechol on slow-wave frequency.

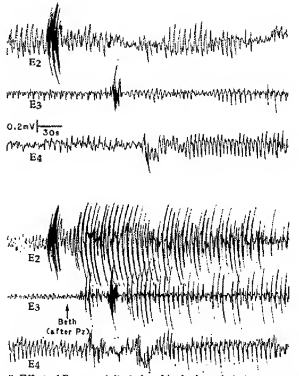

Fig. 7. Effect of Pz on activity induced by bethanechol. *A*: representative MSC immediately after infusion of 10 μg/kg Pz. *B*: 30 min after infusion of 10 μg/kg Pz, 20 μg/kg bethanechol had no effect on MSC propagation but was followed by an increase in spiking activity and a decrease in slow-wave frequency.

circuit characterizing the nervous control and modulation of the MSC is shown in Fig. 9.

The experiments described in this paper took place in awake intact cats; consequently, extraintestinal neural receptors were also stimulated/blocked by the infused pharmacological compounds, and this could account for some of the observed effects. Where possible, systemic loci of action were ruled out; for example, by the use of phentolamine to block sympathetic input during use of McNeil A-343 or bethanechol.

Nicotinic Receptors

On the basis of the inhibitory effect of hexamethonium (Fig. 1), nicotinic receptors appear to be important in the generation of MSC activity. In vitro studies have shown that nicotinic cholinergic receptors are involved in the direct and indirect control of gastrointestinal activity. Stimulation of nicotinic receptors on the postganglionic neurons in both sympathetic and enteric ganglia elicits fast excitatory postsynaptic potentials (EPSPs) (34). Because blockade of sympathetic input by phentolamine to the gut did not significantly affect MSC activity, the nicotinic receptor involved in MSC expression is most likely at the level of the enteric ganglionic nicotinic synapses.

Muscarinic Receptors

Involvement of muscarinic receptors in the MSC was shown by the effects of intraperitoneal or intravenous infusion of atropine. However, both intraperitoneal and intravenous atropine exhibited an unexpected dose-dependent property; low doses were excitatory while high doses were inhibitory. Possible explanations include *1*) atropine at very high doses ($>10^{-4}$ M) has been found to cause excitation of phasic activity in guinea pig ileum in vitro by an unknown direct nonneural mechanism (5, 32). However, in the present experiments, the excitatory dose was lower than the inhibitory dose, whereas guinea pig ileal smooth muscle was inhibited at the lower and excited at the higher dose. *2*) The excitatory action of atropine in the intestine may be analogous to its cardiac action. At low doses, a bradycardia is thought to be caused by an atropine-induced increase in central vagal tone, while high doses cause a tachycardia due to peripheral vagal blockade. The effect of cholinergic blockers that do not cross the blood brain could help determine the involvement of these central receptors in the observed effect of atropine.

A third explanation is suggested based on the heterogeneity of muscarinic receptors. Pharmacological evidence supports the existence of four muscarinic receptor subtypes, two of which are found in the intrinsic nervous

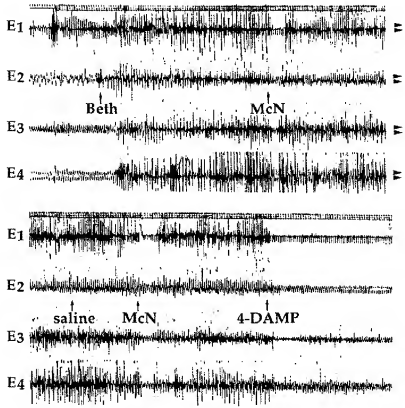

Fig. 9. Effect of serial intravenous infusion of McNeil A-343 (McN) and 4-DAMP on phasic spiking activity induced by bethanechol. All traces are continuous. Bethanechol (20 μg/kg iv) was followed by blockade of MSC propagation, a decrease in slow-wave frequency, and an increase in phasic spiking activity. McNeil A-343 (100 μg/kg) infused 6 min after bethanechol was followed by a transient blockade of spiking activity. Saline had no effect on spiking activity. A second infusion of 100 μg/kg McNeil A-343 repeated 16 min after bethanechol was followed by a transient blockade in spiking activity. 4-DAMP (10 μg/kg) infused 20 min after bethanechol was followed by an immediate and prolonged reduction in spiking activity.

system. M_1 receptors have been found to be involved in a neural inhibitory system governing myoelectric activity of the gastrointestinal tract in several regions, including the esophagus (2), the lower esophageal sphincter (16), the stomach (28), and the small intestine (3, 12, 27). Intracellular recordings of membrane potential of enteric neuronal cell bodies during M_1-receptor stimulation exhibit slow excitatory postsynaptic potentials (24). M_2-subtype muscarinic receptors (20) on the smooth muscle cells mediate contraction in response to postganglionic motoneuron stimulation or exogenous application of muscarinic agonists. Muscarinic receptors located presynaptically on the ganglionic nicotinic synapses decrease neurotransmitter release by functioning as autoinhibitory receptors. The presynaptic muscarinic receptors have been proposed to be identical to the postjunctional smooth muscle M_2-muscarinic receptors (9).

The systemic effects and feedback mechanisms induced by generalized systemic muscarinic blockade may lead to different net results in vivo and in vitro. For example, an increase in synaptic activity due to blockade of autoinhibitory muscarinic receptors at lower doses of atropine than those required to block postjunctional smooth muscle excitatory M_2 receptors could explain the dose-dependent effect. Atropine may also block M_1 receptors in vivo, which would result in excitation of intestinal activity due to a decrease in inhibition. Most reports of the effect of atropine on in vivo intestinal activity have shown only an inhibitory effect (26), although Borody et al. (3) found that intravenous atropine was followed by an

Table 3. *Comparison of effects of saline and α- and β-adrenergic agents on MSC occurrence and propagation velocity after intravenous infusion*

	n	Time to Next MSC After Infusion, min	Duration of MSC Inhibition, min	MSC Period After Infusion or Inhibition, min	Propagation Velocity, mm/s		
					E_1–E_2	E_2–E_3	E_3–E_4
Saline	6	60.8±15.8	60.8±15.8	55.4±8.8	1.8±0.2	2.5±0.1	4.23±0.3
Phentolamine (0.5–1.0 mg/kg)	6	36.7±8.3		45.2±12.6	1.7±0.2	2.3±0.1	4.5±0.4
Phenylephrine (0.1 mg/kg)	6		79.0±14.9				
Oxymetazoline (1–3 μg/kg)	5		126.2±45.5				
Timolol (1 μg/kg)	6	51.8±23.7		47.7±6.1	1.8±0.2	2.4±0.1	4.1±0.3
Isoproternol (30 μg/kg)	6	31.6±15.8		77.7±16.8	2±0.3	2.6±0.1	4.5±0.4

Values are means ± SE; n, number of trials analyzed. No significant changes in any MSC characteristics were noted. Values for average time to next MSC after infusion are for those drugs that did not inhibit propagation of an ongoing MSC. When inhibition of MSC propagation was observed after infusion, interval between blocked MSC inhibition and next MSC to occur was calculated as average duration of MSC inhibition. Saline values for first 2 columns are derived from same MSC interval, that is, first interval after saline infusion.

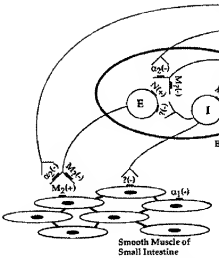

Fig. 9. Proposed locations of receptors involved in expression and regulation of MSC in cat small intestine. I, inhibitory; E, excitatory. *1*) Nicotinic receptors (N) on postganglionic neurons and M_2-muscarinic receptors (M_2) on smooth muscle are most likely active in series in a progressive sloral excitation of intestinal smooth muscle. *2*) M_1-muscarinic receptors (M_1) present on postganglionic neurons are most likely active in an inhibitory circuit that tonically modulates smooth muscle excitability. *3*) α_1-Adrenoceptors (α_1) on smooth muscle and α_2-adrenoceptors (α_2) located presynaptically at ganglionic nicotinic synapse and/or neuromuscular junction may exert a tonic inhibition of MSC frequency, and when stimulated, they inhibit MSC propagation at level of synapse and/or smooth muscle. *4*) β-Adrenoceptors (β) on smooth muscle may also exert a tonic inhibition of MSC frequency; however, their contribution is very weak.

increase in the frequency of MMC in the canine ileum. The initial excitation of MSC activity by the intraperitoneal-infused but not the intravenous-infused atropine may be a concentration-related phenomenon, secondary to a slow increase in tissue atropine concentration following an intraperitoneal compared with an intravenous infusion.

Use of muscarinic agonists and antagonists showed that both M_1- and M_2-muscarinic receptors are involved in the control and modulation of the MSC in cat small intestine. The low-dose/excitatory effect of pirenzepine is best explained by a selective blockade of M_1 receptors in the enteric ganglia. McNeil A-343's P_2-sensitive inhibitory effect was most likely by way of M_1-receptor stimulation; McNeil A-343's action was not blocked by the α-adrenergic antagonist phentolamine, ruling out stimulation of M_1 receptors in sympathetic ganglia as well as a nonspecific α-adrenergic agonist effect by McNeil A-343 (12) as its inhibitory mechanism.

The M_1 receptor involved in MSC inhibition may be at one or both of two enteric neural locations (Fig. 9): *1*) presynaptically, where infusion of an M_1-receptor agonist would decrease and an M_1-receptor antagonist would increase activity at the nicotinic ganglionic synapse and muscarinic neuromuscular junction; *2*) on the cell bodies of postganglionic neurons (PGN) that are part of an inhibitory circuit. Stimulation of these M_1 receptors would result in an increase in EPSP activity in the inhibitory PGN and a subsequent increase in inhibitory input to the smooth muscle and/or other neurons. Location of the M_1 receptor on an inhibitory neuron that has direct input to the smooth muscle is supported by observations in dogs showing that neural ablation by intra-arterial infusion of cobalt (13), or neural blockade by intra-arterial infusion of the nerve blocker tetrodotoxin is followed by an increase in myoelectric activity (26). The transmitter ultimately released by the inhibitory neuron is not known. Evidence supporting a purinergic (8) or a vasoactive intestinal peptidergic (15) identity exists.

Inhibition of MSC propagation following 4-DAMP infusion supports involvement of M_2 receptors, most likely at the smooth muscle postjunctional muscarinic receptor that is activated by motoneuron stimulation (Fig. 9). The M_2-selective agonist bethanechol increased phasic spiking activity by an M_2 receptor-mediated pathway and interrupted MSC propagation by an M_1 receptor-mediated pathway. M_1-receptor stimulation by bethanechol blocked MSC propagation but did not suppress the simultaneous increase in spiking activity; however, M_1-receptor stimulation by McNeil A-343 blocked both MSC propagation and bethanechol-induced phasic spiking. This differential effect was most likely secondary to the specificity of the two pharmacological compounds: *1*) McNeil A-343 acting at M_1 receptors stimulated the inhibitory postganglionic neurons, and possibly the presynaptic autoinhibitory muscarinic receptors, to suppress smooth muscle excitability and neural activity; *2*) high-dose bethanechol also stimulated M_1 receptors, but not as specifically as McNeil A-343; hence, its M_1 receptor-mediated action on the smooth muscle may not have been strong enough to overcome the M_2 receptor-mediated excitation.

M_2-receptor stimulation, by a mechanism that did not involve a sympathetic reflex, also elicited a transient decrease in slow-wave frequency. A decrease in intestinal slow-wave frequency has been observed previously following application of the muscarinic agonist carbachol or during intense spiking (29), possibly by decreasing potassium conductance in the smooth muscle cells (2). A possible explanation of bethanechol's action induced is that the resulting strong contractions compromised the blood flow sufficiently to transiently decrease slow-wave frequency by a metabolic mechanism.

α-Adrenergic Receptors

While nonspecific α-adrenoceptor blockade did not significantly change MSC frequency, stimulation of either α_1- or α_2-adrenoceptors blocked MSC propagation; these receptors may have inhibitory effect on MSC activity. α-Adrenergic input to the gastrointestinal tract has been found to be of extrinsic origin (14). In vitro studies on the guinea pig small intestine show that sympathetic innervation is almost exclusively by presynaptic α_2-adrenoceptors located at the ganglionic nicotinic synapse and at neuromuscular junction muscarinic synapse (23, 25), where it serves to decrease synaptic transmission. Some evidence supports α-adrenergic input directly to the postganglionic neuron cell bodies, although this appears to be

primarily to the submucous rather than the myenteric plexus (22). The smooth muscle cells do not receive direct sympathetic innervation; however, they have α_1-adrenergic receptors that have been proposed to be sensitive to circulating catecholamine and sympathetic overflow from intense sympathetic stimulation in the plexuses (31). Generally, intestinal smooth muscle α-adrenoceptors are inhibitory in nature (6). The α-adrenoceptors involved in the control of the MSC may correspond to the smooth muscle α_1-adrenoceptors and presynaptic α_2-adrenoceptors, although extraintestinal sites of action cannot be ruled out.

β-Adrenergic Receptors

Neither MSC propagation nor frequency was significantly effected by β-acrenoceptor blockade or stimulation. Smooth muscle of the gastrointestinal tract has β-adrenoreceptors that elicit relaxation (7). These β-adrenoreceptors, if present in the feline small intestine, do not appear to be important in the control or modulation of MSC activity.

Conclusion

The preceding pharmacological results show that the MSC is similar to the MMC and GMC observed in other animals in its involvement of nicotinic and muscarinic receptors. One clear difference, however, is in the response to M_1-receptor blockade by Pz; in both cats and dogs, Pz infusion is followed by a shortening of the period of the MSC and MMC; however, in the cat, the MSC propagation velocity is increased while in the dog the MMC propagation velocity is decreased (27).

Migrating complexes (both MSC and MMC) are poorly understood as to mechanism of origin and propagation. That they have never been observed and consequently have never been studied in vitro greatly hampers the ability to selectively study components of the hormonal and neuromuscular milieu in which the in vivo organ functions. However, with a certain degree of caution, some observations made in this paper and in others regarding the involvement of hormones and neuroreceptors in the modulation of migrating complexes can be postulated; the enteric plexus is essential for origin and propagation. Central nervous and hormonal input modulate but are not required for initiation and propagation. Although complexes generally originate in the proximal bowel, any portion of the small bowel can serve as a site of initiation. Migrating complexes are initiated by a different mechanism than that of slow waves and spike bursts and are modulated by different neural input. MSC also influence slow wave-associated spike bursts and slow-wave amplitude, orientation, and frequency.

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Effects of direct and indirect acetylcholine receptor agonists on growth hormone secretion in humans

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Abstract

Cholinergic pathways in the central nervous system positively influence growth hormone (GH) secretion. In fact pyridostigmine, a cholinesterase inhibitor, enhances both basal and GH-releasing hormone (GHRH)-induced GH secretion while, conversely, pirenzepine, an antagonist of muscarinic M_1 receptors, inhibits the GH response to GHRH and to other physiological and pharmacological stimuli. The effect of the cholinergic system on GH secretion probably takes place via inhibition of the release of endogenous somatostatin. In this study in 36 normal adults (26 males and 10 females, age 22–35 years) we compared the effects of three cholinesterase inhibitors (pyridostigmine, 120 mg p.o., $n = 19$; neostigmine, 10 μg/kg i.v., $n = 6$; physostigmine, 12.5 μg/kg i.v., $n = 6$) and bethanechol, a direct muscarinic receptor agonist that is mainly active on muscarinic M_3 receptors (25 μg/kg i.v., $n = 5$), on both basal and GHRH (1 μg/kg i.v.)-stimulated GH secretion. Pyridostigmine, neostigmine and physostigmine induced a significant GH increase (peak vs. basal levels, mean ± S.E.: 10.4 ± 1.6 vs. 0.6 ± 0.2 μg/l, $P = 0.0001$; 13.3 ± 1.2 vs. 0.5 ± 1.1 μg/l, $P = 0.004$; and 14.9 ± 3.1 vs. 2.7 ± 1.1 μg/l, $P = 0.025$;, respectively). These drugs also induced a similar potentiation of the GH response to GHRH (peak: 48.3 ± 5.6 vs. 16.2 ± 2.2 μg/l, $P = 0.0001$; 49.2 ± 2.2 vs. 19.9 ± 5.1 μg/l, $P = 0.006$; and 76.9 ± 12.4 vs. 18.1 ± 5.3 μg/l, $P = 0.001$, respectively). By contrast, bethanechol neither enhanced basal GH secretion (peak vs. basal level: 3.3 ± 1.2 vs. 2.6 ± 1.2 μg/l) nor potentiated the GH response to GHRH (peak: 16.3 ± 1.3 vs. 15.4 ± 1.6 μg/l). The present findings strongly confirm the stimulatory role of acetylcholine on GH secretion in man, showing that indirect acetylcholine receptor agonists stimulate GH secretion, while the muscarinic M_3 agonist bethanechol does not. The results exclude that cholinergic stimulation of GH secretion is mediated by muscarinic M_3 receptors, in keeping with the hypothesis that muscarinic M_1 receptors play the main role in the neuroregulation of GH secretion.

Key words: Growth hormone; Growth hormone-releasing hormone; Cholinesterase inhibitor; Bethanechol

1. Introduction

It is well known that in the central nervous system cholinergic pathways positively influence growth hormone (GH) secretion, both in animals and man. In the latter, antagonists of muscarinic acetylcholine receptors, such as pirenzepine, atropine and methscopolamine have been shown to be capable of suppressing the GH rise elicited by almost all the physiological and pharmacological GH secretagogues, including GH-releasing hormone (GHRH) (Massara et al., 1986; Müller, 1987). On the other hand, indirect cholinomimetics cholinesterase inhibitors, such as edrophonium (Leveston end Cryer, 1980) and pyridostigmine (Massara et al., 1986; Ghigo et al., 1987), enhance basal GH secretion. Moreover, pyridostigmine enhances the GH response to GHRH (Massara et al., 1986; Ghigo et al., 1987), without sex-related differences (Arvat et al., 1993). The effect of cholinergic modulation on GH secretion probably takes place via inhibition of endogenous hypothalamic somatostatin release (Richardson et al., 1980; Locatelli et al., 1986; Casanueva et al., 1986; Torsello et al., 1988; Wehrenberg et al., 1992).

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In this study we compared the effects on both basal and GHRH-stimulated GH secretion of pyridostigmine with those of neostigmine and physostigmine, two other cholinesterase inhibitors, and bethanechol, a direct acetylcholine receptor agonist.

2. Materials and methods

Thirty-six healthy volunteers (26 males and 10 females, age 22–35 years, body mass index 20–22 kg/m^2) were studied after informed consent was obtained. None of the subjects suffered from organic or psychiatric illnesses. All subjects were non-smokers and no additional medication was allowed before and during the entire study. Four studies were performed, in each of which every subject underwent 3 tests in random order and at least 2 days apart.

Study 1 ($n = 19$): (1) GHRH (GHRH-(1–29), Novabiochem, Laufelfingen, Switzerland; 1 μg/kg administered as i.v. bolus at time 0 min); (2) pyridostigmine (pyridostigmine bromide, Mestinon, Hoffmann-La Roche, Basel, Switzerland; 120 mg administered p.o. at time −60 min); (3) pyridostigmine + GHRH.

Study 2 ($n = 6$): (1) GHRH (1 μg/kg administered as i.v. bolus at time 0 min); (2) neostigmine (neostigmine methilsulphate, Prostigmine, Hoffmann-La Roche, Basel, Switzerland; 10 μg/kg administered as i.v. bolus at time 0 min); (3) neostigmine + GHRH.

Study 3 ($n = 6$): (1) GHRH (1 μg/kg administered as i.v. bolus at time 0 min); (2) physostigmine (physostigmine salicilate, S.A.L.F., Bergamo, Italy; 12.5 μg/kg administered as i.v. bolus at time 0 min); (3) physostigmine + GHRH.

Study 4 ($n = 5$): (1) GHRH (1 μg/kg administered as i.v. bolus at time 0 min); (2) bethanechol (bethanechol chloride, Urecholine, Merck, Sharp and Dohme, Rahway, NJ, USA; 25 μg/kg infused i.v. over 5 min, from time −5 to 0 min); (3) bethanechol + GHRH. The dose of bethanechol was chosen on the basis of a pilot study, in which 50 μg/kg of the drug induced overt peripheral muscarinic side effects. It has to be noted that this bethanechol dose was unable to modify basal GH levels.

All tests were performed after an overnight fasting. An indwelling catheter was inserted in an antecubital vein and kept patent by a slow infusion of saline. Blood samples were drawn every 15 min from time −60 to 90 min in testings with pyridostigmine alone and from time −15 to 90 min in all other tests.

Serum GH levels were measured in duplicate by immunoradiometric assays (HGH-CTK Irma, Sorin, Saluggia, Italy). All samples from an individual subject were analysed at the same time. The sensitivity of the assay was 0.15 μg/l. The inter and intra-assay coefficients of variation were between 2.3 and 5.5% and between 1.9 and 3.9%, respectively.

The results were expressed as means ± S.E., either of absolute values (basal levels or absolute peaks, μg/l) or of the area under the GH response curve (AUC, μg/l/h), calculated by trapezoidal integration from time 0 to 90 min. Statistical analysis was performed by using non-parametric (Kruskall-Wallis) analysis of variance and Wilcoxon's signed rank test where applicable.

3. Results

The results are reported in Table 1 and Fig. 1.

Basal GH levels did not differ significantly in different tests.

Oral administration of pyridostigmine increased basal GH levels with mean peak levels occurring at 30 min (peak vs. baseline: $P = 0.0001$) and enhanced the

Table 1
Growth hormone (GH) responses to growth hormone-releasing hormone (GHRH) and cholinergic agonists administered either alone or in combination

Study	Test	Basal	Peak	AUC
(1)	GHRH	0.7 ± 0.3	16.2 ± 2.2	604.9 ± 106.3
	Pyridostigmine	0.6 ± 0.2	10.4 ± 1.6	351.9 ± 59.9
	Pyridostigmine + GHRH	1.2 ± 0.3	48.3 ± 5.6	2023.2 ± 239.8
(2)	GHRH	1.6 ± 0.8	19.9 ± 5.1	814.8 ± 208.6
	Neostigmine	1.2 ± 0.5	13.3 ± 1.5	504.9 ± 44.2
	Neostigmine + GHRH	5.6 ± 3.0	49.2 ± 2.2	1960.4 ± 133.3
(3)	GHRH	0.9 ± 0.6	18.1 ± 5.3	664.4 ± 228.5
	Physostigmine	2.7 ± 1.1	14.9 ± 3.1	533.1 ± 123.2
	Physostigmine + GHRH	3.2 ± 1.2	76.9 ± 12.4	3278.1 ± 531.3
(4)	GHRH	0.7 ± 0.4	15.4 ± 1.6	520.9 ± 59.4
	Bethanechol	2.6 ± 1.2	3.3 ± 1.2	108.1 ± 44.8
	Bethanechol + GHRH	1.4 ± 0.9	19.5 ± 2.0	608.0 ± 63.7

Data are shown as mean ± S.E. of basal and peak levels (μg/l) and area under curve (AUC, μg/l/h).

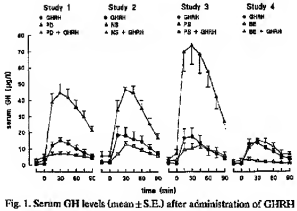

Fig. 1. Serum GH levels (mean ± S.E.) after administration of GHRH and cholinergic agonists, administered either alone or in combination. The panels correspond to the studies performed with pyridostigmine (PD), neostigmine (NS), physostigmine (PS) and bethanechol (BE).

GH response to GHRH, both when evaluated as peak ($P = 0.0001$) and as AUC ($P = 0.0001$).

Intravenous administration of neostigmine increased basal GH levels with mean peak levels at 30 min (peak vs. baseline: $P = 0.004$) and enhanced the GH response to GHRH, both when evaluated as peak ($P = 0.006$) and as AUC ($P = 0.003$).

Intravenous administration of physostigmine increased basal GH levels with mean peak levels at 45 min (peak vs. baseline: $P = 0.025$) and enhanced the GH response to GHRH, both when evaluated as peak ($P = 0.001$) and as AUC ($P = 0.001$).

In contrast, intravenous administration of bethanechol failed to increase both basal and GHRH-stimulated GH secretion.

Pyridostigmine, neostigmine and physostigmine induced similar GH increases. In addition, all these drugs exerted a true potentiating effect (evaluated as AUC) on the GH response to GHRH. In fact the effect of combined administration of the anticholinesterase and GHRH was higher than the sum of the effect of the two substances administered alone ($P = 0.0002$, $P = 0.048$ and $P = 0.016$, for pyridostigmine, neostigmine and physostigmine, respectively).

3.1. Side effects

GHRH induced a transient facial flushing in 20/36 subjects. Pyridostigmine, neostigmine and physostigmine induced mild peripheral cholinergic side effects, i.e. abdominal pain, diplopia, dysarthria and muscular fasciculations in 15/19, 5/6 and 4/6 subjects, respectively. Physostigmine also induced a mild sense of discomfort in 3/6 subjects. Bethanechol induced abdominal pain and mild sweating in all subjects.

When comparing the GH responses of subjects who experienced side effects with those of subjects who did not, no difference was recorded.

4. Discussion

In this study in young healthy volunteers, intravenous administration of neostigmine and physostigmine released GH and potentiated the GH response to GHRH, effects that were superimposable on those previously reported for p.o. administered pyridostigmine (Massara et al., 1986; Ghigo et al., 1987). The potentiating effect of physostigmine seemed to be more evident, though not statistically different, among the cholinesterase inhibitors. However, the different routes of administration of the drugs and the limited number of subjects do not allow us to draw conclusions. In contrast to the cholinesterase inhibitors, the direct muscarinic receptor agonist bethanechol had no effect on either spontaneous or GHRH-stimulated GH secretion.

Acetylcholine is known to play a crucial role in GH secretion in both experimental animals and man (Leveston and Cryer, 1980; Massara et al., 1986; Ghigo et al., 1987; Müller, 1987). Experimental data have been presented suggesting that the effect of cholinergic modulation of GH secretion takes place through inhibition or stimulation of hypothalamic somatostatin release. In fact, (1) acetylcholine inhibits somatostatin release from rat hypothalamus (Richardson et al., 1980); (2) procedures able to abolish somatostatinergic tone, such as cysteamine pretreatment (Locatelli et al., 1986), anterolateral deafferentation of mediobasal hypothalamus (Locatelli et al., 1986) and pretreatment with somatostatin antibodies (Torsello et al., 1988; Wehrenberg et al., 1992) blunt the effect of cholinergic and anticholinergic drugs on GH secretion; and (3) atropine does not modify basal and GHRH-induced GH secretion by rat pituitary cells in culture (Casanueva et al., 1986).

Viewed in this context, the discrepancy between the effectiveness of cholinesterase inhibitors and the lack of effect of bethanechol in stimulating GH secretion may be explained considering that the former unselectively stimulate all subtypes of muscarinic receptors as well as nicotinic ones, while bethanechol is reportedly mainly active on muscarinic M_3 receptors with only a small effect on M_1 and M_2 ones (Taylor, 1985; Mei et al., 1991). In fact, studies performed with pirenzepine, which is a relatively selective antagonist of muscarinic M_1 receptors (Mei et al., 1989; Hulme et al., 1990), suggest that muscarinic receptors of the subtype M_1 are involved in the neuroregulation of GH secretion. The present data therefore reinforce the view that muscarinic M_1 receptors are involved in the neuroregulation of GH secretion. A similar conclusion has been

reached recently by Locatelli et al. (1993), who have shown in conscious dogs that of the muscarinic receptor subtypes, M_1 was the one principally involved in the control of GH secretion via somatostatin. Along this line, it has to be noted that arecoline, a direct acetylcholine receptor agonist mainly active on muscarinic M_2 receptors (Vanderheyden et al., 1990), has no effect on basal GH levels (Nurnberger et al., 1983). In partial disagreement with all these data, there is the observation that RS-86 does not enhance basal GH secretion (Casanueva et al., 1989). This drug has been proposed as a muscarinic M_1 receptor agonist, although there is little evidence of its selectivity (Palacios et al., 1986; Palacios and Spiegel, 1986) and the drug has been abandoned for clinical use as a centrally active muscarinic M_1 receptor agonist.

The possibility cannot be ruled out that the GH response to inhibition of cholinesterase activity may be mediated, at least in part, by activation of nicotinic acetylcholine receptors, which in humans has been reported to stimulate GH release (Mendelson et al., 1981). In fact, one could hypothesize that the increased availability of acetylcholine in the synaptic cleft, due to cholinesterase inhibitors, is further enhanced by the effect of the neurotransmitter on positive presynaptic nicotinic autoreceptors. Such receptors have been demonstrated in cholinergic synapses in the central nervous system (Araujo et al., 1990).

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